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Cantharocybe virosa, first record of the genus in Thailand

Jaturong Kumla, Nakarin Suwannarach, Saisamorn Lumyong*

Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

* Correspondence to: *saisamorn.l@cmu.ac.th*

Abstract—Mushroom specimens collected in Thailand were identified as *Cantharocybe virosa* based on morphology and similarities of LSU and ITS genes. A full description, color images, and line drawing are provided. This is the first record of the genus *Cantharocybe* from Thailand.

Key words—*Agaricales*, gill fungus, *Hygrophoraceae,* taxonomy

Introduction

Cantharocybe (*Hygrophoraceae*, *Agaricales*) was described by Bigelow & Smith (1973) with *C. gruberi* (A.H. Sm.) H.E. Bigelow & A.H. Sm.as the type species. This genus has a worldwide distribution in temperate and tropical regions. Three species of *Cantharocybe* have been described: *C. brunneo-velutina*, *C. gruberi*, and *C. virosa*; they are saprotrophic and usually grow on soils (Bigelow & Smith 1973, Manimohan & al. 2010, Ovrebo & al. 2011, Kumar & Manimohan 2013). Although no species of *Cantharocybe* has been reported from Thailand (Chandrasrikul & al. 2011), during a taxonomic survey of macrofungi collected in northern Thailand, we found specimens that corresponded to the description of *C. virosa*, previously reported from Bangladesh and India (Manimohan & al. 2010, Kumar & Manimohan 2013, Hosen & al. 2016, Acharya & al. 2017). Here we describe and illustrate the morphological characteristics of the Thai material and cite supporting evidence from LSU and ITS gene sequence matches in GenBank.

Materials & methods

Morphology studies

Basidiocarps were collected from the campus of Chiang Mai University, Chiang Mai Province, Thailand, and wrapped in aluminum foil or kept in plastic collection boxes until transported back to the laboratory; notes on macromorphological features and photographs were taken within 24 h of collection. Color names and codes follow Kornerup & Wanscher (1978). The micromorphological data were derived from dried specimens mounted in 95% ethanol followed by distilled water, 3% KOH, or Melzer's reagent. Dimensions of anatomical features were from at least 50 measurements of each structure. The specimens were dried at 40–45 °C on an electric food dryer and deposited in the herbarium of Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA was extracted from a fresh specimen using a Favorgen DNA Extraction Mini Kit following the manufacturer's instructions. The large subunit (LSU) region of ribosomal DNA (rDNA) was amplified by polymerase chain reaction (PCR) with LROR/LR5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. The internal transcribed spacer (ITS) rDNA region was amplified using ITS4/ITS5 primers under the previously listed thermal conditions. Negative controls lacking fungal DNA were run to check for reagent contamination. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using a Macherey-Nagel NucleoSpin® Gel and PCR Clean-up Kit following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was performed by 1ST Base Company in Kembangan, Malaysia using the same PCR primers mentioned above. Sequences were used to query GenBank database via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html).

Taxonomy

Cantharocybe virosa (Manim. & K.B. Vrinda) T.K.A. Kumar,
Mycotaxon 124: 235 (2013). Fig. 1

Pileus 30–85 mm diam., convex, becoming broadly convex; surface light brown (6D5) to grayish brown (7E3) or dark grey (7F8), slightly darker at the centre; margin inrolled when young, becoming incurved and finally becoming straight. Lamellae adnate to decurrent, <1.5 mm wide, white (4A1) to yellowish white (1A2). Stipe 30–60 × 5–20 mm, central or at times slightly excentric, solid, cylindrical or tapering towards the base, concolorous with the pileus, with cottony mycelium at the base. Odor strong and unpleasant. Spore print white.

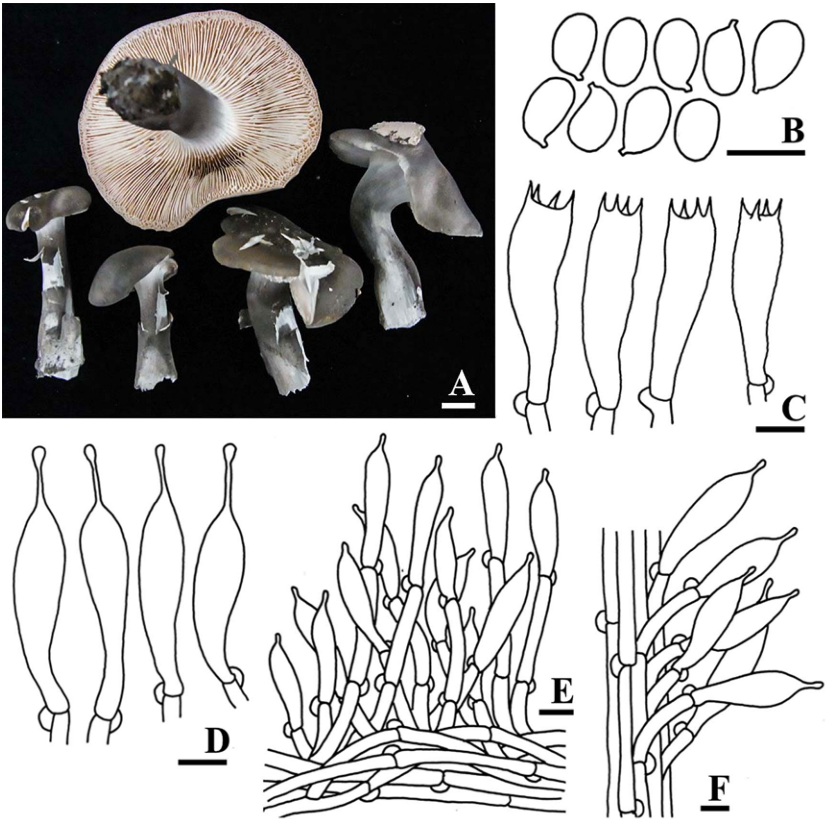

Fig. 1. *Cantharocybe virosa* (SDBR-CMU-M0145). A: basidiocarps; B: basidiospores; C: basidia; D: cheilocystidia; E: trichoderm pileipellis; F: surface of stipe in longitudinal section showing caulocystidia. Scale bars: A = 10 mm, B–F = 10 µm.

Basidiospores 6–11 × 5–7 µm (n = 50), Q = 1.2–1.7, subglobose to ellipsoid, thin-walled, smooth, with refractive guttules, inamyloid. Basidia 22–55 × 6–12 µm, clavate, 4-spored, with basal clamp connections, sterigmata <5 µm. Pleurocystidia absent. Cheilocystidia abundant, 20–65 × 5–9 µm, lecythiform to lageniform, sometimes with a mucronate apex, base portion usually clavate, the upper portion extending into an elongated neck <35 µm long with or without a rounded capitulum, thin-walled, hyaline to pale yellow. Lamellar trama parallel to sub-regular, composed of branching hyphae 3–15 µm diam., septate, thin-walled, hyaline to pale yellow. Pileipellis a trichoderm, interwoven,

composed of hyphae 4–10 µm diam., septate, thin- to slightly thick-walled, often pale brown vacuolar to plasmatic pigment; terminal cells 20–60 × 3–10 µm, cylindrical to narrowly clavate; pileocystidia with or without extending neck, with one or two short rounded capitula, elongated neck <15 µm long. Stipitipellis composed of branching hyphae 2–10 µm diam., septate, thin- to slightly thick-walled, outer surface more or less covered with cylindrical to narrow clavate cells 30–100 × 6–10 µm, with or without a rounded capitulum head, pale brown vacuolar to plasmatic pigments. Clamp connections present in all tissue types.

Specimens examined—**THAILAND**, **Chiang Mai Province**, **Muang District**, Chiang Mai University, 18°48′00″N 98°57′21″E, elevation 334 m, on soil, 4 September 2015, Kumla J. & Suwannarach N. (SDBR-CMU-M0145; GenBank MG694689, MG694691); 4 October 2017 Suwannarach N. (SDBR-CMU-NK0280, GenBank MG694690, MG694692).

Molecular analysis

The LSU and ITS sequences of specimens SDBR-CMU-M0145 and SDBR-CMU-NK0280 were deposited in the GenBank database. Our LSU sequences (MG694689, MG694690) were 100% similar to the *C. virosa* TENN 63483 ex-paratype sequence JX101471; and our ITS sequences (MG694691, MG694692) were 99% similar to the *C. virosa* TENN 63483 ex-paratype sequence KX452405.

Discussion

The clitocybeoid light brown to dark grey basidiocarps, abundant lecythiform or mucronate cheilocystidia, and the subglobose to ellipsoid smooth inamyloid basidiospores support placement of our collections in *Cantharocybe* (Bigelow & Smith 1973, Lodge & al. 2014). The two specimens collected in northern Thailand were initially identified as *C. virosa* after consulting the descriptions by Kumar & Manimohan (2013) and Acharya & al. (2017) and keys by Kumar & Manimohan (2013) and Hosen & al. (2016). The pale yellow to lemon yellow pileus and narrowly elliptical to oblong (11–17.5 × 4.5–7.5 µm) basidiospores of *C. gruberi* (Bigelow & Smith 1973) clearly distinguish that species from *C. virosa*. *Cantharocybe brunneovelutina* Lodge & al. differs from *C. virosa* by its velutinous basidiocarps, cheilocystidia with multiple prong-like appendages at the apex resembling a basidia-like structure, and shorter (9–9.5 × 5.5–6 µm) basidiospores (Ovrebo & al. 2011). The LSU and ITS molecular analyses support the morphological differences (Hosen & al. 2016).

Geographical distributions also support three species: *C. virosa* has been reported in tropical southern Asia (Bangladesh; India) and is a known toxic species (Manimohan & al. 2010, Kumar & Manimohan 2013, Hosen & al. 2016, Acharya & al. 2017). *Cantharocybe gruberi* is a widely distributed northern temperate species recorded from western North America (British Columbia; western U.S.A.) and Europe (Spain) (Bigelow & Smith 1973, Hosen & al. 2016). *Cantharocybe brunneovelutina* is known only from its type locality in tropical Central America (Belize; Ovrebo & al. 2011).

The ITS and LSU sequence matches in GenBank confirmed that the two Thai specimens belong to *C. virosa*. The combination of morphological and molecular characteristics supports the identification of *C. virosa*, a new record in Thailand.

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